Identifying The Prognostic Significance of Mitochondrial AAA Proteases YME1L1 Expression in Ovarian Cancer

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Research Article

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Abstract

Background:

The discovery of early diagnosis and prognostic markers for ovarian cancer can significantly improve survival and reduce mortality. The role of the YME1L1 signaling axis in genetic alterations and immune infiltration of the tumor microenvironment remains unclear.

Methods:

Bioinformatics web resources, including GEPIA2, cBioPortal, Oncomine, Kaplan–Meier Plotter and TIMER were used to analyze the expression profile, prognostic value and immune infiltration of YME1L1.

We further performed tissue microarray analysis of paraffin-embedded tissues from 60 ovarian cancer patients recorded at FIGO/TNM cancer staging.

Results:

Here, we use multi-omics analysis of multiple histological data to map the role of epigenetic and genetic alterations of YME1L1 in tumor immune infiltration and prognosis of cancer patients. We explored YME1L1 gene expression profiles by systematically analyzing the association of YME1L1 expression with the prognosis of ovarian cancer patients confirmed in multiple databases. High YME1L1 expression was associated with poorer overall survival and disease-free survival associated. Together, our studies suggest that YME1L1 may modulate tumor survival, immune features and contribute to tumor immune invasion, poor prognosis, and immunotherapy failure.

Conclusion:

Our findings may have clinical implications for the design of treatment strategies, prognosis assessment and follow-up management of patients receiving immunotherapy in a variety of cancers.

Introduction

Ovarian cancer is the second most common gynecological malignancy after cervical cancer, which is also the most fatal of all female reproductive cancers. The poor prognosis is due to most diagnoses occurring in stages III or IV and lack of effective screening methods which could reduce the mortality rate of ovarian cancer (1). The symptoms of ovarian cancer including abdominal bloating, discomfort in the abdomen or pelvic area, fatigue, weight loss, poor appetite and frequent urination (2). The indistinct symptoms often lead to misdiagnosis or delayed diagnosis. According to the research, the overall five-year survival rate for advanced ovarian cancer is less than 50%. Approximately one-fourth of women lose their lives within 90 days of being diagnosed with advanced ovarian cancer, and more than 40% died in the first year of diagnosis(3, 4)[33][39]. Of the various types of ovarian cancer, epithelial ovarian cancer

accounts for 95%, the remainder types of ovarian cancer are germ cell tumors and sex cord stromal tumors (3).

Mitochondria are membrane bound organelles present in almost all eukaryotic cells, and perform multiple functions for eukaryotic cell survival. These activities are organized and maintained by numerous of proteins encoded in the nuclear and mitochondrial genomes. Faulty regulation of mitochondrial proteostasis may damage the function of organelles and jeopardize health.

In recent years, there are increasing studies illuminating the oncogenicity of AAA ATPase family. The overexpression of certain AAA protease was correlated with advanced stage, tumor depth, tumor growth, lymph node metastasis, and distant metastasis (5-9). Due to the AAA ATPase family is involved in multiple cellular processes, including regulation of gene expression, mitochondrial dynamics, signal transduction and cell death. As such, AAA family is a promising drug target in cancer treatment (7, 10).

YME1L was identified as the human orthologue of the Yme1p subunit of yeast mitochondrial i-AAA protease (11). It is an ATP-dependent metalloprotease and a member of the highly conserved AAA family, which means YME1L is ATP-driven and embedded in the inner mitochondrial membrane (12).

The aim of this study is to explore the expression of YME1L1 gene in ovarian cancer through several clinicopathological databases, so as to understand the possible relationship between YME1L1 and ovarian cancer. To determine the genetic variation of YME1L1 in ovarian cancer tissues compared with other tumor samples, we investigated the expression pattern of YME1L1 in ovarian cancer tissues by using cBioPortal database. The immune infiltration relationship between YME1L1 and ovarian cancer tumor was analyzed through the TIMER databases.

Our results provide new concepts that could improve the prognostic accuracy of OV and highlight the relevance of YME1L1 and immune cell infiltration that could divide OV patients into favorable and unfavorable risk groups that benefit from current immunotherapy. We further explore the relationship between genetic alterations, immune cell infiltration, and precision therapy.

Materials And Methods

2.1. ONCOMINE

We used previous methods to screen the oncogene microarrays from the Oncomine database on a large scale, finding outliers, predicting co-expressed genes, etc (13). We retrieved the mRNA expression data of YME1L1 gene from the database in tumor tissues and normal tissues. In our analysis, $p < 0.05$, 2-fold change and top 10% of gene classes were set as thresholds.

2.2. cBioPortal

We used cBioPortal to mine gene set data and ovarian cancer gene variants. Gene co-occurrences in YME1L1 mutations, copy number variants (CNV) and ovarian cancer were analyzed by the cBioPortal

tool. mRNA expression was z-scored against all samples (log RNA Seq V2 RSEM) with a z-score threshold set at ±2.

2.3. GEPIA2

Gene Expression Profiling Interactive Analysis (GEPIA) is an interactive network database that can be linked and analyzed with other databases (TCGA and GTEx). Using GEPIA, we analyzed 9736 tumors and 8587 normal tissues (14, 15). The database provides interactive and customizable features including differential expression analysis, spectral mapping, correlation analysis, survival analysis, and genetic analysis.

2.4. Tissue microarray (TMA) and immunohistochemistry (IHC) analysis

IHC staining and scores were the same as in previous studies. The method is briefly described for tissue array slides (CJ2) of human ovarian cancer and non-tumor tissues purchased from SuperBioChips Laboratories (Seoul, Republic of Korea). For immunohistochemical (IHC) assays and scoring, the methods were performed as described in (16). Slides were treated with antibodies. All slides were digitized with high accuracy at ×40 (0.26 um/pixel) using a Motic Easyscan digital slide scanner (Motic Hong Kong Limited, Hong Kong, China). Motic Easyscan full slide images were viewed using DSAssistant and EasyScanner software at the Li-Tzung Pathology Laboratory (Kaohsiung, Taiwan).

2.5. Human Protein Atlas

The Human Protein Atlas is a publicly available database containing mRNA and immunohistochemistry (IHC)-based protein expression data from 17 different forms of human cancer. It allows researchers to create a database of information on protein expression patterns in a given type of tumor. In this study, IHC images were used to compare the expression of different YME1L1-containing protein genes between normal and OV tissues.

2.6. TIMER

We analyzed the same methods as previously published (17), using TIMER to explore the association between the expression of different YME1L1 genes and the abundance of immune infiltrates in ovarian cancer. We also analyzed the correlation between YME1L1 expression and genetic markers of tumor-infiltrating immune cells.

2.7. Statistical Analyses

To assess differences in the expression of YME1L1 clinicopathologically defined groups, Student's t test for differential significance and chi-square test for categorical variables were used. A p-value < 0.05 was considered to indicate a statistically significant difference. GraphPad Prism (GraphPad software, La Jolla, CA, USA) was utilized to analyze the statistical differences by using t-test or Fisher's exact test for two groups and one-way ANOVA for multiple group comparisons. Kaplan–Meier curves were plotted to

study survival tendency, and the p value was estimated using the log-rank test. Statistical significance, *p value < 0.05; **p value < 0.01; ***p value < 0.001.

Results

3.1. Identification of YME1L1 mutated genes in ovarian cancer

First, we found that YME1L1 levels were much higher in ovarian cancer only in pan-cancer than in other cancer tissues after analysis by the Oncomine database (Figure 1A). Significant changes in YME1L1 gain and loss were observed in the copy number variation ratio distribution and box plot (Figure 1B). Analysis of the frequency of concurrent gene changes in YME1L1 gene alterations by the cBioPortal database revealed a total of 1748 genes with concurrent gene changes that were enriched in both the YME1L1-altered and unaltered cohorts (Figure 1C-D). We assessed the mutational load of each type of ovarian cancer by counting the mutations in each tumor sample. Most of the ovarian cancers had mutation load within the range of variation (Figure 1E).

3.2. Genetic variation of YME1L1 in different tumors

Next, we used the cBioPortal database to evaluate the type and frequency of YME1L1 alterations in OV tissues based on sequencing data from YME1L1 patients obtained from TCGA's Pan-Cancer Atlas database (Figure 2A). The genetic alterations of YME1L1 in various tumor types in the TCGA dataset were then investigated using cBioPortal. We found that OV tumor samples had the highest YME1L1 genetic amplification frequency (red block), which was the most altered tumor among all TCGA tumor samples. It is worth mentioning that endothelial carcinoma of YME1L1 presented high mutation rate in pan-cancer (Figure 2B). As shown in Figure 2C, a total of different YME1L1 variants were detected in TCGA tumor samples, including missense mutations, truncation mutations, fusion mutations and in-frame mutations. residues 400-700 of the YME1L1-encoded protein have many mutation sites, making it the most frequently mutated region of the YME1L1 protein.

3.3. Survival and expression of YME1L1 in OV and normal tissues

We used TNM plots to analyze YME1L1 expression from RNA-seq data (p = 4.79e-03). We found that YME1L1 levels in egg cancer were much lower than normal tissues (Figure 3A). Next, we evaluated YME1L1 expression according to different clinical stages and we found that YME1L1 expression was significantly increased in both tumor tissue and advanced patients (Figure 3B). We analyzed the effect of YME1L1 on overall survival and progression-free survival in OV patients using Kaplan-Meier plots. Our results indicated that high YME1L1 expression was associated with poor prognosis (Figure 3C&D). We further analyzed YME1L1 protein levels using tissue microarray (TMA) from the Human Protein Atlas (HPA). In OV patients, we found that YME1L1 showed moderate staining in OV patients respectively (Figure 3E). We also analyzed the sensitivity and specificity of YME1L1 in OV and the results showed that the expression of selected genes in the tumor samples was higher than the percentage of normal samples at each major cut-off value. Figure 3F shows an example output of the normal tumor

comparison. To verify the role of YME1L1 in ovarian cancer, we verified the expression of YME1L1 in different types of ovarian cancer using the Oncomine dataset. The results showed that YME1L1 mRNA levels were significantly higher in ovarian serous cystadenocarcinoma and ovarian carcinoma compared to normal tissues in different databases (Figure 4). Overall, our findings suggest that YME1L1 upregulation is highly associated with ovarian cancer and that YME1L1 plays an important role in tumor cancer progression.

3.4. YME1L1 Expression in Ovarian Cancer Tissue Microarray Analysis

To further confirm the accuracy of the multi-component analysis, we evaluated YME1L1 detected in tumor tissues using a commercial ovarian tissue microarray (TMA) using immunohistochemistry. the results of YME1L1 expression in ovarian cancer tissues in IHC staining are shown in Figure 5A. The IHC fraction of YME1L1 increased significantly with increasing stage (Figure 5B&C). This now occurred in the cytoplasm of YME1L1, while no statistical difference was observed in the nucleus ($p < 0.05$). On the other hand, YME1L1 expression was higher in the cytoplasm in malignant tumors than in benign tumors. There was no significant difference in YME1L1 expression in the nucleus (Figure 5D&E). The results of our analysis are consistent with the above-mentioned multi-omics database, with higher levels of YME1L1 expression in late stages.

3.5. Association of YME1L1 gene with immune infiltration in ovarian cancer

To further explore the immune-related functions of YME1L1, we analyzed the association between YME1L1 and the tumor microenvironment in TIMER. YME1L1 expression was significantly associated with the presence of immune cells (Figure 6A). Interestingly, we found that high levels of YME1L1 mRNA expression were associated with high immune infiltration in ovarian cancer. YME1L1 mRNA expression levels were positively correlated with many immune cells, including: monocyte (LRP: $r = 0.396$, CD86: $r = 0.042$, CD163: $r = 0.168$), T cells (IFNGR1, $r = 0.21$, IL12RB2: $r = 0.185$, IL17RB: $r = 0.214$) , B cells (CD148: $r = 0.281$, CD93: $r = 0.255$, CXCR4: $r = 0.242$) and macrophages (NR3C2: $r = 0.123$, TLR4: $r = 0.149$, LAMP2: $r = 0.368$). In addition, B cells and neutrophils were found to increase with YME1L1 mutation compared to wild-type YME1L1 (Figure 6C). In this study, a significant correlation was found between YME1L1 expression and infiltration levels of monocyte, T cells, B cells and macrophages in ovarian cancer. These findings strongly suggest that YME1L1 plays an important role in the immune infiltration of ovarian cancer.

3.6. Potential drugs targeting YME1L1 by pharmacogenomic screening

We further retrieved potential drugs targeting YME1L1 from the pharmacogenetic database to find potential drugs for OV. Figure 7A shows that 2 of the 476 drugs of molecular importance are NSC319726 and SB216763, which promote and suppress the expression of YME1L1, respectively. We targeted SB216763 ($r = -0.269$), which has the ability to reduce the level of YME1L1 in multiple ovarian cancer cell lines. We found a high sensitivity and negative correlation ($p = 0.003$) between SB216763 and YME1L1.

The results indicated that SB216763 was effective in inhibiting the expression of YME1L1 in multiple ovarian cancer cell lines (Figure 7C).

Discussion

Ovarian cancer is a heterogeneous disease that includes several types of tumors with distinct clinicopathological and molecular features and prognosis. The majority of cases of ovarian cancer are of epithelial origin, which has been classified into 5 histological subtypes: serous, the most common; endometrioid; mucinous; clear cells and Brenner tumors. Besides, undifferentiated carcinomas or mixture type are present in some cases (3). The standard treatment is surgery followed by platinum-taxane chemotherapy but still with poor long-term survival rate. Regarding to the fact that ovarian cancer is among the deadliest of gynecological malignancies, there has been a surge to explore more effective targeted therapies are required to boost survival rates for women with ovarian cancer (18-20).

The main physiological function of mitochondria is to provide energy for cell proliferation and cell growth through oxidative phosphorylation. In addition, mitochondria are responsible for cell metabolism, differentiation and apoptosis by regulating redox reactions, amino acid and lipid metabolism, reactive oxygen species (ROS) production, calcium homeostasis and mitochondrial permeability transition pore (mPTP) (21, 22). Mitochondrial dysfunction is closely related to tumorigenesis, growth, invasion and metastasis (23). Mitochondria are capable of performing a series of biologically precise functions with specialized molecular partners, the mitochondrial unfolded protein response (mtUPR) and processing peptidases that facilitate proper protein folding and complex assembly. A set of endogenous mechanisms maintains the dynamic balance of mitochondrial protein homeostasis (24).

Inner membrane is the mainly location where mitochondrial process of oxidative phosphorylation and generate ROS with abundant protein studded on it. Two forms of mitochondrial AAA proteases, m-AAA and i-AAA protease, are considered as quality control enzymes in the inner membrane. i-AAA proteases is composed of homologous YME1L1 subunits, which form cylinder-shaped hexameric complexes, exposes its catalytic domains to the intermembrane space. In contrast to i-AAA, m-AAA protease faces its catalytic domains to the matrix side with a composed of a homo-oligomeric AFG3L2 or a hetero-oligomeric form of AFG3L2 and paraplegin (25-29).

Cristae are invaginations protruding into the mitochondrial matrix and studded with respiratory complexes and ATP synthase. The folding structure increase surface area for oxidative phosphorylation. The dynamin-like GTPase OPA1 is one of protein complexes that maintain the shape of cristae, which responsible for inner membrane fusion and maintenance of cristae structure by bridging apposing membranes in the cristae fold (30). It was also identified as a substrate of YME1L1. The membrane-anchored OPA1 is cleaved by proteases OMA1 and YME1LI at S1 and S2 respectively to generate short variant OPA1 (S-OPA1), which lacks the membrane-anchoring domain. The uncleaved OPA1 is considered as OPA1 long form (L-OPA1), the form required for inner-membrane fusion (31, 32). However, the optimal fusion depends on the balance of fusion-active L-OPA1 and fusion-inactive S-OPA1 isoforms. Thus,

YME1L may mediate mitochondrial fusion, respiration, and cristae morphology maintenance through processing OPA1(32, 33).

The YME1L1 gene is also believed to be related to the mechanism of NUMT insertion. The transfer of DNA fragments from mitochondrial to the nuclear DNA (NUMTs) is a natural biological phenomenon in eukaryotes and plays an important role in genomic variability (34). In the process, different insertion loci may have completely different effects on cells. The insertion of NUMTs is non-random, the inferred insertion points of NUMTs have a strong associated with specific chromatin regions and with retrotransposons(35). In some cases, NUMT is related to aging, mutations and oncogenesis (31, 36). For yeast *Saccharomyces cerevisiae,* it is well known that inactivation Yme1p causes an increased frequency of mtDNA escape from mitochondria to the nucleus (37-39). In a recent study, human YME1L1 reduces the escape of mtDNA into the nucleus when expressed in an inactive Yme1 yeast strain. The finding indicated that YME1LI may be a NUMT suppressor gene in humans, whose inactivation results in increased numtogenesis (39).

So far, the correlations between YME1L1 and clinicopathological data and patient survival had not yet been investigated in ovarian cancers. Our study demonstrated that the expression levels of YME1L1 were elevated in ovarian cancer tissues compared with other tumor tissues, and YME1L1 mRNA levels were significantly higher in ovarian carcinoma tissues compared to normal tissues. In general, the mutational load of ovarian cancer is within the range of variation, while the most mutant sites are in residues 400-700 of the YME1L1-encoded protein. The survival analysis indicated that overexpression of YME1L1 was associated with lower survival rate in women with ovarian cancer, the result was in line with the significantly increased of YME1L1 expression in advanced cancer stage patients. Overall, our findings emphasized the upregulation of YME1L1 is highly associated with ovarian cancer and tumor progression.

Only few research have been investigated the relationship between YME1L1 and cancer, relatively, which is a topic worthy of further exploration. YME1L1 was identified as one of the seven gliomas landscape genes, which cooperatively promote gliomagenesis and contribute to poor overall survival and prognosis (40). Reprogramming of mitochondria enables cells to respond to environmental variations and challenges such as hypoxic conditions during tumorigenesis. Through the axis of mTORC1–LIPIN1–YME1L, cells perform mitochondrial proteostasis between mitochondrial dynamics and cell metabolism. YME1L downgrades lipid transfer proteins and mitochondrial protein translocases to manipulate dynamics of mitochondria and support cell development. YME1L-mediated mitochondrial reshaping helps the cell growth of pancreatic ductal adenocarcinoma (PDAC). When cells are present in hypoxic conditions, as in the environment of solid tumors like PDACs (41), YME1L may be regulated by a HIF1α-dependent reaction, and rewiring of glutamine metabolism was observed. YME1L-mediated proteolysis with of stabilization of HIF1α may help tumor growths and adjust mitochondrial function in harsh environments (42).

It is established that ovarian cancers are highly hypoxia-dependent solid tumor. Under hypoxia condition, highly expression of HIF-1α in epithelial ovarian cancer helps tumor cells to endure hypoxia increased

tumor tumor growth and metastasis (43, 44). mTOR also play an important role in ovarian cancer progression, especially mTORC1 activity is required for cell proliferation (45). To the present, there are still further studies and evidences needed to demonstrate whether the modulating mechanism to promote the growth of PDAC of YME1L1 gene can be repeated on ovary cancer cells

Conclusion

In conclusion, our findings provide different information regarding the importance of YME1L1 in carcinogenesis and its potential role as a diagnostic and prognostic biomarker for ovarian cancer. Therefore, molecularly targeted therapeutic strategies to inhibit YME1L1, a key target of the granulosa and its signaling pathway, are expected to be the focus of cancer research. Subsequently, experiments will be conducted to further explore the correct molecular mechanisms of YME1LI in cancers of interest.

Declarations

Author Contributions: Writing—original draft preparation, W.-T.L., C.-J.L.; Visualization, P.-Y.C., C.-C.S., M.-S.H., Y.-T.C. and W.-L.T.; writing—review and editing, C.-C.W. and C.-J.L.; supervision, C.-J.L.; funding acquisition, C.-J.L. All authors have read and agreed to the published version of the manuscript.

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for open access to the database

Availability of data and materials: The datasets generated and analyzed during this study are available in the

TCGA, GTEx, ImmPort and Timer database.

Ethics approval and consent to participate: Not applicable.

Competing interests: No potential conflicts of interest.

Consent for publication: Not applicable.

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Figures

Figure 1

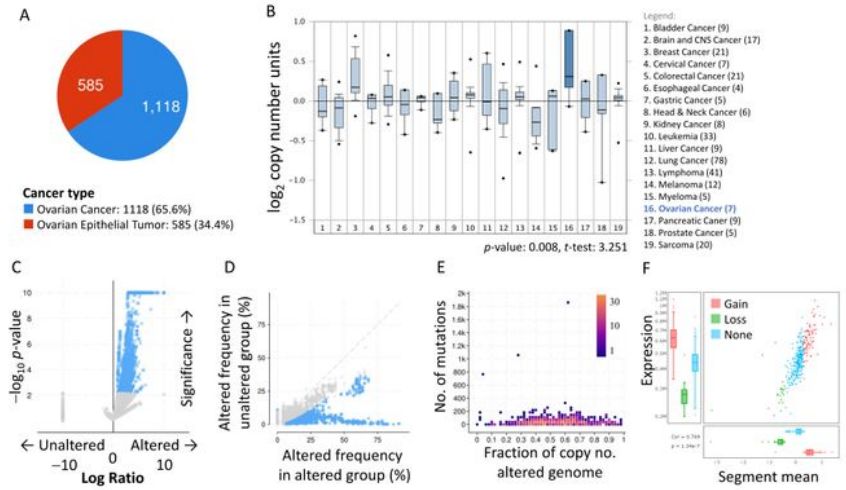

Figure 1

Expression and variation of YME1L1 in ovarian cancer. (A) Differential expression of YME1L1 mRNA in pan-cancer. (B) Distribution and correlation of copy number variation were labeled as gains and losses. (C & D) Volcano plot and scatter plot showing genes associated with frequency changes of YME1L1 copy number alteration. (E) Heatmap provides the distribution and correlation of YME1L1 mutations with copy number alteration in ovarian cancer.

Figure 2

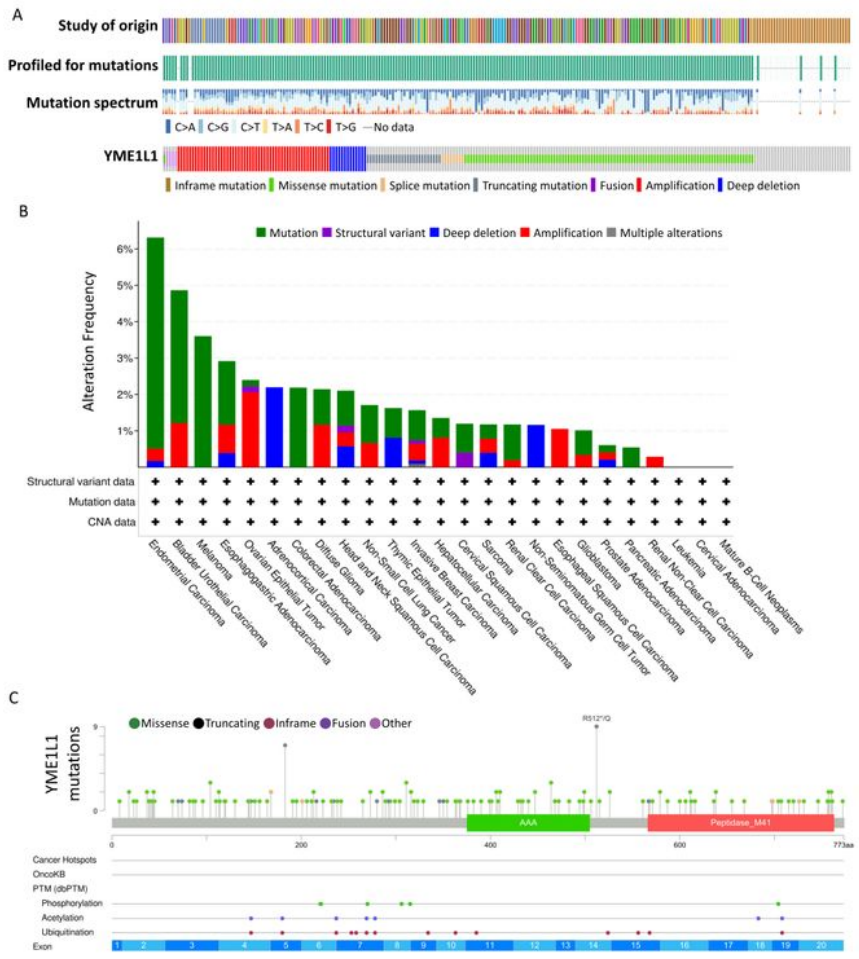

Figure 2

Frequency and type of YME1L1 alterations in ovarian cancer. (A) Analysis of various mutations in the YME1L1 gene in human cancer data. (B) Genetic alterations of YME1L1 gene in various cancer types using cBioPortal cancer genomics analysis. (C) YME1L1 protein domain map showing specific mutation sites.

Figure 3

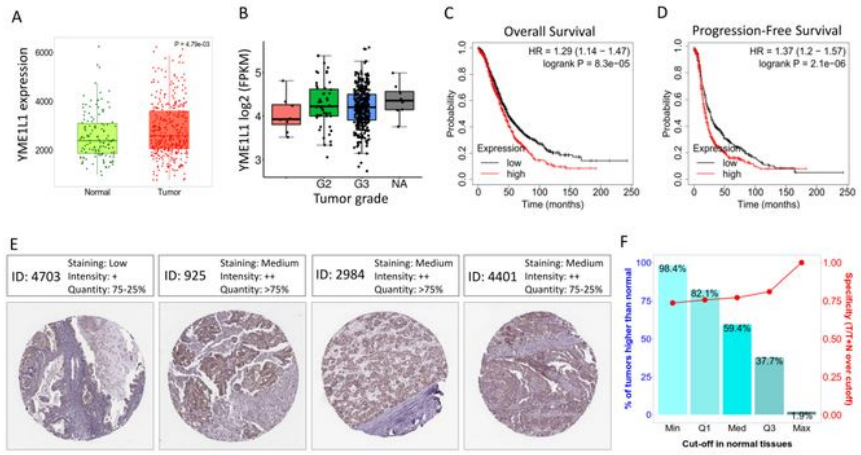

Figure 3

Relative expression and survival of YME1L1 in ovarian cancer tissues based on multiple databases. (A) Expression of YME1L1 in ovarian cancer and normal tissues. (B) Box plot of YME1L1 mRNA expression in ovarian cancer patients based on tumor grade assessment. (C&D) Overall survival and progress-free survival estimates of YME1L1 mRNA levels from the Kaplan-Meier plotter database. (E) Representative

images of YME1L1 IHC staining in ovarian cancer from the human protein atlas dataset. (F) Box plots and bar graphs of YME1L1 gene expression from RNA sequencing data and gene microarray data.

Figure 4

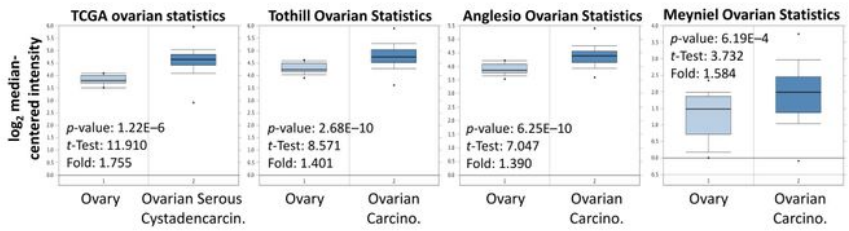

Figure 4

Box plot of YME1L1 mRNA levels in ovarian cancer and normal tissues from Oncomine data. p<0.05 indicates statistical significance; YME1L1 is one of the top 10% overexpressed genes in all four different datasets of OV.

Figure 5

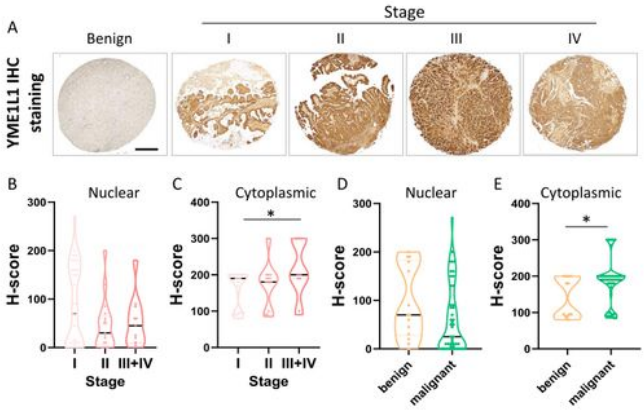

Figure 5

Protein levels of YME1L1 in ovarian cancer. (A) Representative images of YME1L1 expression in ovarian cancer tissues at benign and different stages of staining. (B-D) The expression level of YME1L1 in the nucleus and cytoplasm is evaluated in the violin chart of ovarian cancer at different stages, benign and malignant. * p < 0.05. Scale bar = 500 mm.

Figure 6

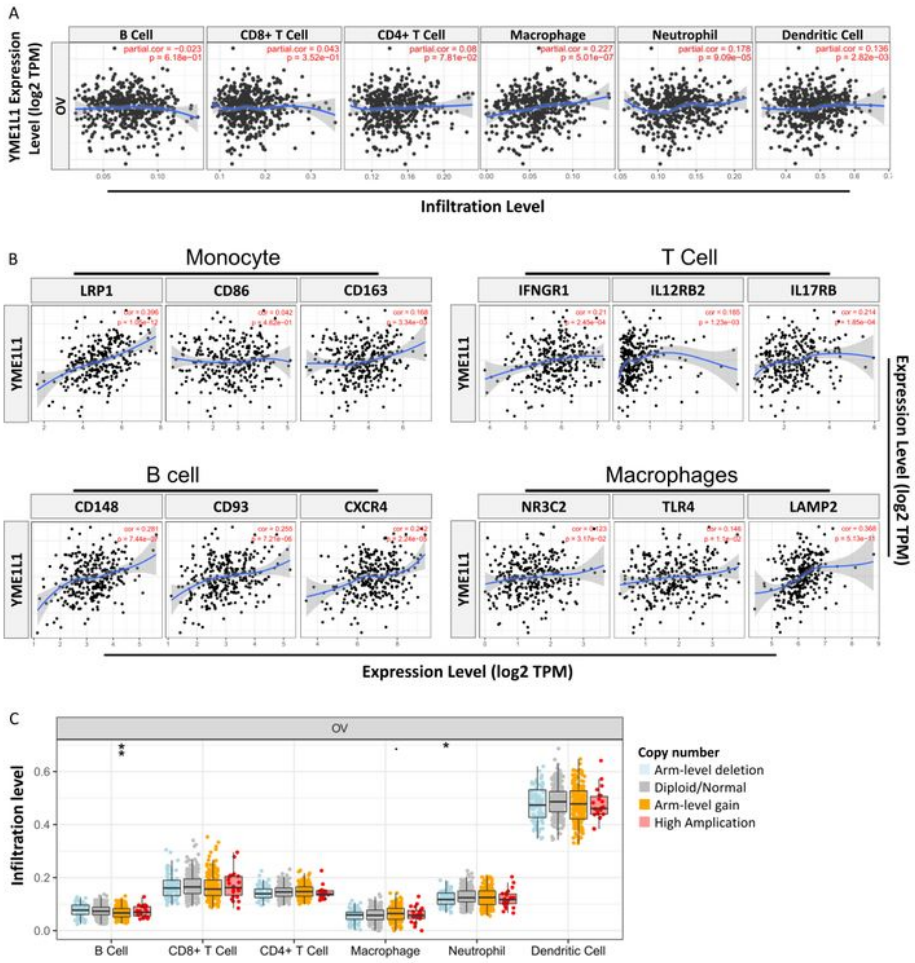

Figure 6

Correlation between CNV, immune cell infiltration and OV prognosis of YME1L1. (A) Correlation between YME1L1 transcript levels and immune infiltration. (B) Relationship between YME1L1 and various genetic markers in monocytes, T cells, B cells and macrophages in ovarian cancer. (C) Infiltration levels of various immune cells in ovarian cancer at different YME1L1 copy numbers. * p < 0.05, ** p< 0.01.

Figure 7

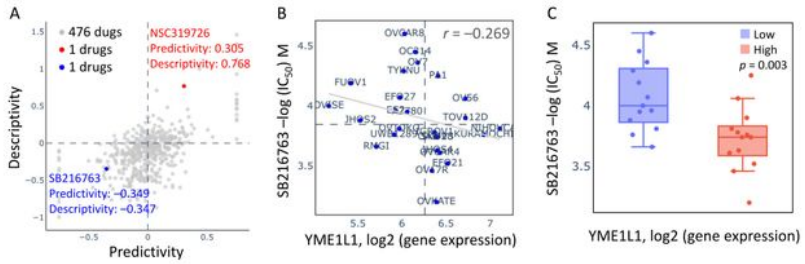

Figure 7

Screening for drug sensitivity in ovarian cancer cells with high YME1L1 expression. (A) YME1L gene characterization and screening of potential drugs through pharmacogenomic database. (B) Drug sensitivity of YME1L gene to SB216763 in multiple ovarian cancer cell lines. (C) Efficacy of YME1L in SB216763 to inhibit ovarian cancer cells.